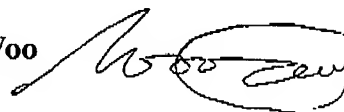ENGLISH TRANSLATION OF

KOREAN PATENT APPLICATION NO. 10-2003-0019069

APPLICATION NUMBER: 10-2003-0019069
FILING DATE: March 27, 2003
APPLICANT: Seoul National University Industry Foundation

I AM A TRANSLATOR OF HMP, AND DECLARE THAT I PREPARE THIS TRANSLATION AND IT IS TRUE ENGLISH TRANSLATION MADE FROM KOREAN PATENT APPLICATION NO. 10-2003-0019069.

At Seoul, Korea on April 10, 2006

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Abstract

The present invention relates to a gene controlling the root growth of plants and a method for enhancing the root growth of plants using the same, and more particularly to a root growth controlling gene, *GmEXP*1, isolated from soybean (*Glycine max*), an expansin protein encoded by the gene and a method for enhancing root growth of plants by overexpression of the gene in the plants. The *GmEXP1* gene of the present invention and the expansin protein expressed from the *GmEXP1* gene can be utilized in the characteristic improvement for root growth of plants and in the investigation of root growth regulating genes in other plants. Also, the *GmEXP1* gene plays important roles in accelerating development of plants by enhancing the root growth.

Representative Drawing

Figure 15

Index word

Root development, gene, expansin

GENE CONTROLLING ROOT GROWTH OF PLANTS AND METHOD FOR ENHANCING ROOT GROWTH OF PLANTS USING THE SAME

Brief Description of the Drawings

5   FIG. 1 shows the results of the multiple sequence alignment of the base sequences of a *GmEXP1* gene and α-expansin genes according to the present invention.

FIG. 2 is a phylogenetic profile of the *GmEXP1* gene according to the present invention.

FIG. 3 shows the results of a Southern blot analysis of the *GmEXP1* gene

10   present in the soybean genome (A: hybridization with a probe containing only the coding region of the *GmEXP1* gene, B: hybridization with a *GmEXP1* gene-specific probe containing the coding region and the 3'-untranslated region of the *GmEXP1* gene, RV: digested with *Eco*RV, H: digested with *Hin*dIII, N: digested with *Nco*I).

FIG. 4 is the Northern blot analysis results showing the expression patterns of

15   the *GmEXP1* gene according to a kinds of soybean organs.   25S rRNA is an internal control (A: leaf, B: stem, C: hypocotyl and D: root).

FIG. 5 is the Northern blot analysis results showing the expression patterns of the *GmEXP1* gene at different developmental stages of soybean root.   25S rRNA is an internal control.

20   FIG. 6 is a graph showing the growth patterns of the primary, secondary and tertiary roots in soybean seedlings (□: primary root, ■: secondary root and ▲: tertiary root).

FIG. 7 is the Northern blot analysis results showing the expression patterns of the *GmEXP1* gene in the primary and secondary roots of 5-day old soybean seedlings.

25S rRNA is an internal control (A: whole roots, B: primary root and C: secondary root).

FIG. 8 is the Northern blot analysis results showing the spatial expression patterns of the *GmEXP1* gene in soybean roots.   25S rRNA is an internal control (A: diagram of sections of the whole root of 5-day old soybean, B: results of Northern blot performed on the sections of A, C: diagram of sections of the whole root of 9-day old soybean, D: results of Northern blot performed on the sections of C).

FIG. 9 is the in situ hybridization analysis results showing the spatial expression patterns of the *GmEXP1* gene using longitudinal sections of the primary root of soybean (A: hybridization with the antisense probe for the *GmEXP1* gene, B: hybridization with the sense probe for the *GmEXP1* gene, C: enlarged image of the region of elongation in A, rc: root cap, e: epidermis, m: meristem, c: cortex, v: vascular cylinder, bars in A and B: 250 μm, bars in C: 100 μm).

FIG. 10 is the in situ hybridization analysis results showing the spatial expression patterns of the *GmEXP1* gene using cross sections of the primary root of soybean. (A: region that is 80 μm apart from the root tip, B: region that is 1.0 mm apart from the root tip, C: region that is 1.3 mm apart from the root tip, D: region that is 4.0 mm apart from the root tip, rc: root cap, e: epidermis, m: meristem, c: cortex, v: vascular cylinder, bars in A: 50 μm, bars in B, C and D: 100 μm).

FIG. 11 is the in situ hybridization analysis results showing the expression patterns of the *GmEXP1* gene at the secondary root initiating stage (A: longitudinal section hybridized with the antisense probe, B: cross section hybridized with the antisense probe, C: cross section hybridized with the sense probe, lri: secondary root initial, e: epidermis, v: vascular cylinder, c: cortex, bars: 100 μm).

FIG. 12 shows the anatomical features of transgenic tobacco plants

overexpressing the *GmEXP1* gene, which are observed by a microscope (A: cross

section of a leaf of wild-type tobacco, B: cross section of a leaf of transgenic tobacco,

C: cross section of the stem of wild-type tobacco, D: cross section of the stem of

transgenic tobacco, E: cross section of the petiole of wild-type tobacco, F: cross section

5   of the petiole of transgenic tobacco, e: epidermis, pp: palisade parenchyma, sp: spongy

parenchyma, c: cortex, x: xylem, p: pitch, bars in A and B: 300 μm, bars in C and D:

1000 μm, bars in E and F: 100 μm).

FIG. 13 shows the root apical region of transgenic tobacco plants

overexpressing the *GmEXP1* gene, which are observed by a microscope (left: wild-type

10   tobacco, right: transgenic tobacco, bars: 100 μm).

FIG. 14 is a graph comparing the length of wild-type tobacco root grown under

acid condition with the length of transgenic tobacco root where the *GmEXP1* gene is

overexpressed (A: wild-type tobacco, B; transgenic tobacco, C: transgenic tobacco, □:

pH 7.0, ■:pH 4.5).

15   FIG. 15 is a graph comparing the length of wild-type tobacco root receiving the

obstacle-touching stress with length of transgenic tobacco root where the *GmEXP1* gene

is overexpressed   (A: wild-type tobacco, B; transgenic tobacco, C: transgenic tobacco,

□:no obstacle-touching stress, ■: obstacle-touching stress).

20   **Detailed description of the invention**

Object of the invention

Technical field and background of the invention

The present invention relates to a gene controlling the root growth of plants and

a method for enhancing the root growth of plants using the same, and more particularly

to a root growth controlling gene, *GmEXP*1, isolated from soybean (*Glycine max*), an

expansin protein encoded by the gene and a method for enhancing root growth of plants

by overexpression of the gene in the plants.

5

A root is a plant organ that has adapted to acquire water and nutrients from the

environment (Schiefelbein J.W. et al., *Plant Cell*, 9:1089-1098, 1997).   A root consists

of epidermis, cortex and vascular tissues in radial rows or concentric circles (Esau K.,

Anatomy of Seed Plants, Ed. 2, 215-245, 1997; Dolan L. et al., *Development*, 119:71-84,

10   1993; Raven P.H. et al., *Biology of Plants*, 6th Ed. Worth Publishers, New York, 1999).

In the longitudinal section, a root can be divided into three different regions; cell

division, elongation and maturation region (Dolan L. et al., *Development*, 119:71-84,

1993; Baluska F. et al., *Plant Physiol.*, 112:3-4, 1996).   The region of cell division

carries out new cell divisions.   The cells derived from the region of cell division

15   expand and elongate mostly in the region of elongation.   The elongated cells begin to

differentiate in a region of maturation, where root hairs and secondary roots are initiated.

The cell elongation and maturation in a root are controlled by the extensibility of cell

wall and the turgor pressure inside the cell (Cosgrove D.J., *BioEssays*, 18533-540,

1996).

20

However, it is known that the extent of plant cell elongation is confined by cell

walls.   The cell wall is composed of polysaccharides, proteins, phenolic compounds

and other materials (Varner J.E. et al., *Cell*, 56:231-239, 1989).   The plant cell wall

plays a determinative role in establishing the size and shape of a plant cell.   For

elongation or maturation, however, a plant cell needs to selectively modify its cell wall. The agents for cell wall modification in the plant cell include various cell wall components, such as expansins, endoglucanases, xyloglucan endotransglycosylases and hydroxyl radicals (Cosgrove D.J., *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 50:391-417, 1999; Cosgrove D.J., *Plant Physiol. Biochem.*, 38:109-124, 2000a; Cosgrove D.J., *Nature*, 407:321-326, 2000b).

Expansins are generally considered as primary agents for cell wall elongation (Vissenberg K. et al., *Plant Cell*, 12:1229-1237, 2000). Expansins cause wall creep by loosening hydrogen bonds between cellulose microfibrils and matrix polymer (McQeen-Mason S. et al., *Proc. Natl. Acad. Sci.* USA, 91:6574-6578, 1994; Cosgrove D.J., *Plant Cell*, 9:1031-1041, 1997). Since the first cloning of an expansin gene (Shcherban T.Y. et al., *Proc. Natl. Acad. Sci.* USA, 92:9245-9249, 1995), many expansin genes have been identified from a variety of plant species. And they are known to form a multigene family (Cosgrove D.J., *Plant Physiol.*, 118:333-339, 1998). The expansin genes are classified into three subfamilies, α-, β- and γ-expansin subfamilies, based on their phylogenetic relationship (Li Y. et al., *Plant Physiol.*, 128:854-864, 2002). The α-expansins compose a major portion of the expansins, including the ones from tomato (*Lycopersicon esculentum*) (Keller E. et al., *Plant J.*, 8:795-802, 1995), rice (*Oryza sativa*) (Cho H-T. et al., *Plant Physiol.*, 113:1137-1143, 1997a), oat (*Avena sativa*) (Li Z-C et al., *Planta*, 191:349-356, 1993) and Arabidopsis (*Arabidopsis thaliana*) (Cosgrove D.J., *Plant Physiol.*, 118: 333-339, 1998; Li Y. et al., *Plant Physiol.*, 128:854-864, 2002). The α-expansin subfamily can be further divided into A, B, C and D groups (Link B.M. et al., *Plant Physiol.*, 118:907-916, 1998).

Expression patterns of the α-expansin genes have been extensively studied in deepwater rice and tomato. It was reported that the transcript of an expansin gene in deepwater rice, *OsEXP4*, increases in abundance before onset of cell wall extensibility

5   and faster growth, supporting the role of expansins in cell elongation (Cho H-T et al., *Plant J.*, 15:805-812, 1998). Also, in tomato, expression of the Le*EXP18* gene was localized in a group of cells in the shoot apical meristem where incipient leaf primordium initiation takes place (Reinhardt D. et al., *Plant Cell*, 10:1427-1437, 1998). International Patent Publication No. WO02086066 discloses a novel β-expansin

10   polypeptide for modifying the structure of cell walls in a plant and a nucleotide sequence encoding the same. U.S. Patent No. 5,929,303 discloses a fruit-specific and ripening regulation expansin gene.

Studies of a variety of expansin genes and their tissue-specific expression

15   patterns show that different expansin genes may play different roles in various cell types during organ development in plants (Rose et al., *Proc. Natl. Acad. Sci*, USA, 94:5955-5950, 1997). Therefore, it is still necessary to study new expansin genes and their functions.

20   Accordingly, while studying new expansin genes, the inventors have conducted isolation of root-specific *GmEXP*1 gene from soybean and discovered that root growth of plants can be controlled by the *GmEXP* 1 gene.

Technical Problem

An object of the present invention is to provide a novel protein capable of controlling root growth of plants.

Another object of the present invention is to provide a gene encoding the

5   protein.

Still another object of the present invention is to provide a recombinant vector comprising the gene.

Still another object of the present invention is to provide bacteria transformed with the vector.

10   Still another object of the present invention is to provide a method for enhancing root growth of plants using the gene.

Still another object of the present invention is to provide a method for enhancing the resistance in plants to obstacle-touching stress using the above gene.

Still another object of the present invention is to provide a method for selecting

15   a root growth controlling gene or protein using the above gene or protein.

Still another object of the present invention is to provide a method for identifying a compound affecting the activity or expression of the above protein, comprising the steps of culturing the protein or a recombinant cell expressing the protein and a candidate material and measuring an effect on the activity or expression of

20   the protein.

Composition and Function of the invention

To achieve the above objects, the present invention provides a novel protein

capable of controlling root growth of plants.

In another aspect, the present invention provides a gene encoding the protein, wherein the gene is expressed root-specific manner in plants.

In another aspect, the present invention provides a recombinant vector

5   comprising the gene and bacteria transformed with the vector.

In another aspect, the present invention provides a method for enhancing root growth of plants using the gene.

In another aspect, the present invention provides a method for enhancing the resistance in plants to obstacle-touching stress using the above gene.

10   The above method is characterized in that root growth of a plant is enhanced or resistance in a plant to obstacle-touching stress is improved by overexpression of the gene in the plant.

In another aspect, the present invention provides a method for selecting a root growth controlling gene or protein using the above gene or protein.

15   In another aspect, the present invention provides a method for identifying a compound affecting the activity or expression of the above protein, comprising the steps of culturing the protein or a recombinant cell expressing the protein and a candidate material and measuring an effect on the activity or expression of the protein. The above compound is characterized by enhancing the activity or expression of the inventive

20   protein.

Hereinafter, the present invention will be explained in more detail.

To isolate new expansin genes, the inventors designed probes targeted to a

common conserved region of generally known expansin genes and isolated one positive

clone through investigation of a cDNA library of soybean.   Upon analysis of the

nucleotide sequence and amino acid sequence of the isolated clone, it was discovered

that the cDNA isolated from soybean consists of 1,089bp with an open reading frame

5   encoding a polypeptide of 255 amino acids.

A gene isolated from soybean according to the present invention shows high-

level of sequence homology to the group of α-expansins including tobacco, cucumber,

rice and Arabidopsis, (See FIG. 1).   Therefore, the inventors termed this gene

10   "*GmEXP1*" and registered its nucleotide sequence with the GenBank (Accession No.

AF516879).   According to the phylogenetic classification, the *GmEXP1* gene of the

present invention belongs to Group D of the α-expansin subfamily (See FIG. 2).

In one embodiment of the present invention, a Southern blot analysis was

15   performed to confirm whether the *GmEXP1* gene is actually present in the soybean

genome.   The analysis results show that a variety of expansins having homology to the

α-expansin are present in the soybean genome, and that the *GmEXP1* gene of the

present invention is present in a single copy (See FIG. 3).

20   It is generally known that expansin genes are expressed in various regions of

plants at different developmental stages.   Most expansin transcripts are most abundant

in actively growing organs, such as leaf primordia in tomato, internodes in rice, pollen

in maize and soybean, pistil in tobacco, fruits in strawberry and tomato, and coleoptiles

in oat, implying that the expansin genes are involved in critical developmental processes

in plants.

In another embodiment of the present invention, to investigate the expression patterns of the *GmEXP1* gene in various organs of soybean, Northern blot analysis was preformed. The analysis results show that the *GmEXP1* gene of the present invention is only expressed specifically in the soybean root (See FIG. 4). Based on the assumption that the *GmEXP1* gene is closely related to the root growth of soybean, a Northern blot analysis was performed to detect the expression patterns of the *GmEXP1* gene in roots at different developmental stages. It was revealed that the *GmEXP1* gene is expressed in all developmental stages of the root (See FIG. 5) and the expression of the *GmEXP1* gene is up-regulated particularly when soybean roots elongate rapidly (See FIG. 6 and FIG. 7).

Further, the spatial expression patterns of the *GmEXP1* gene in soybean roots were analyzed in more detail by Northern blot and in situ hybridization. The results show that the *GmEXP1* gene is highly expressed in the root tip involved in cell division and elongation in the primary and secondary roots. Also, the results show that the *GmEXP1* gene is highly expressed in the secondary root initials emerging from the primary root. However, the *GmEXP1* gene was not detected in the region where cell division had ceased (See FIG. 8).

According to the in situ hybridization, the *GmEXP1* gene is expressed in the regions undergoing cell division and elongation in soybean roots and shows expression patterns specific to the epidermis and underlying cell layers of the region of elongation

(See FIGs. 9 and 10). This suggests that the expression of gene according to the invention is related to the root development in the plant.

In still another embodiment of the present invention, in situ hybridization was conducted to identify the relationship between the *GmEXP1* gene and the initiation of root formation and the role of the *GmEXP1* gene in the root development of plants. It was revealed that the *GmEXP1* gene is highly expressed in the epidermis of the primary and secondary root initials and in the tip region of the emerging secondary root (See FIG. 11). According to the analysis results, the *GmEXP1* gene of the present invention is expressed in the root-specific pattern and plays an important role in the root growth of plants.

The inventors obtained transgenic plants overexpressing the *GmEXP1* gene by transforming different species of plants with the *GmEXP1* gene. In one example of the present invention, transgenic plants overexpressing the *GmEXP1* gene were obtained by introducing the *GmEXP1* gene into tobacco plants (*Nicotiana tabacum*) by *Agrobacterium tumefaciens*-mediated transformation. Also, the change of phenotypes of the transgenic plants was observed. It was observed that the transgenic plants overexpressing the *GmEXP1* gene are relatively bigger than wild-type plants. The leaves and xylem cell layers in the stems of the transgenic plants were thicker than those of the wild-type plants (See FIG. 12). Also, the root tips of the transgenic plants were more elongated than those of the wild-type plants (See FIG. 13). It is clear that the *GmEXP1* overexpression accelerates the root growth of plants.

However, the expansin genes are known to have an activity regulated by various environmental stresses. For example, it was reported that low water potential increases the expansin activity (Wu et al., *Plant Physiol.*, 111:765-772, 1996). Thus, the inventors incubated transgenic plants with the *GmEXP1* gene introduced thereto

5   under both the neutral and acidic condition and compared the growth level of the transgenic plants with that of the wild-type plants. The transgenic plants overexpressing the *GmEXP1* gene exhibited rapid root growth under both the neutral and acidic condition, as compared to the wild-type plants (See FIG. 14).

10   When plants are grown in soil, their roots may encounter obstacle-touching stress. Under obstacle-touching stress, the Arabidopsis roots begin to bend to realign themselves (Okada K. et al., *Science*, 250:274-276, 1990). In one embodiment of the present invention, the reaction of the transgenic plants overexpressing the *GmEXP1* gene against the obstacle-touching stress was observed. The results show that the

15   transgenic plants exhibited rapid root growth even under the obstacle-touching stress as compared to the wild-type plants, without showing any difference from the control plants, which were not under the obstacle-touching stress (See FIG. 15). This implies that the *GmEXP1* overexpression provides the transgenic plants with insensitivity to the obstacle-touching stimulus, and that the gene plays an important role in overcoming the

20   obstacle-touching stress when plants grow in soil.

In another embodiment of the present invention, which compares the epidermal cell length of the transgenic tobacco roots with that of the wild-type tobacco, it can be confirmed that the epidermal cells in the transgenic tobacco roots are longer than those

in the wild-type tobacco roots.   In transgenic plants overexpressing the *GmEXP1* gene, root epidermal cells are rapidly elongated, thereby resulting in the rapid growth of the plants.

5   Accordingly, the present invention provides a novel protein controlling the root growth of plants. The range of protein according to the present invention comprises a protein comprising an amino acid sequence set forth in SEQ ID No. 2, and functional equivalents thereof. The "functional equivalents" refer to protein as a result of addition, substitution and deletion of amino acid having more than 70%, preferably more than

10   80%, most preferably more than 90%, sequence homology to the amino acid sequence of SEQ ID No. 2 and exhibiting substantially the same physiological activity of the protein of SEQ ID No. 2.   Also, "substantially the same physiological activity" means an activity enhancing root growth of plants when a protein is overexpressed in the plants. The protein according to the present invention can be obtained from nature (for

15   example, plant cells) or by expression of a recombinant nucleic acid encoding the protein or by a chemical synthesis.   Preferably, the protein can be isolated from soybean.

          Additionally the present invention is provided a gene controlling root growth,

20   *GmEXP1* gene, encoding the above protein.   Preferably, the gene is represented by SEQ ID NO: 1. The root growth control gene of the present invention has high homology to the genes which belong to the α-expansin subfamily.   According to phylogenetic classification, the *GmEXP1* gene of the present invention belongs to Group D of the α-expansin subfamily.   The *GmEXP1* gene is expressed in a root-

specific manner and preferentially expressed in the regions of cell division and
elongation of plant roots.

The root growth controlling gene of the present invention is inserted into a

5   suitable expression vector to transform suitable plant cells. The "expression vector"

refers to a known plasmid, virus or other vehicle known in the art that has been

manipulated by insertion or incorporation of the *GmEXP*1 genetic sequences. The

nucleic acid sequence of the present invention can be operably linked to the expression

control sequence. The operably linked nucleic acid sequence and expression control

10   sequence can be included within a single expression vector containing a selective

marker and a replication origin. The "operably linked" may mean that a nucleic acid

sequence and an expression control sequence are linked in such a manner to enable

expression of the nucleic acid. The "expression control sequence" refers to the nucleic

acid sequence that regulates the expression of a nucleic acid sequence to which it is

15   operably linked in a particular host cell. Such a control sequence includes promoters

for performing transcription, operator sequences for controlling transcription, sequences

encoding a suitable mRNA ribosome- binding site, and sequences controlling

termination of transcription or translation. A suitable vector into which the *GmEXP1*

gene can be introduced is a Ti plasmid, a root inducing (Ri) plasmid or a plant virus

20   vector. The most suitable vector may be, but not limited to, a binary vector of pPZP,

pGA or pCAMBIA series. Anyone skilled in the pertinent art can select a suitable

vector for introducing the nucleic acid of the present invention. Any vector capable of

introducing the *GmEXP1* gene sequence into plant cells can be used in the present

invention. One example of the present invention describes a pGA643/*GmEXP1*

recombinant vector that introduces the *GmEXP1* gene into pGA643 vector including
CaMV 35S promoter.

The recombinant vector according to the present invention can be introduced
5   into plant cells by known methods which include, but not limited to, transformation
using *Agrobacterium* species, particle gun bombardment, silicon carbide whiskers,
sonication, electroporation and PEG (polyethyleneglycol) precipitation. Accordingly,
the present invention provides host cells transformed with the inventive vector. The host
cells are preferably bacteria, such as *E.coli.* or *Agrobacterium* species.

10   The present invention also provides a method for enhancing root growth of
plants using the *GmEXP1* gene.

The present invention also provides a method for enhancing the resistance in
plants against the obstacle-touching stress using the *GmEXP1* gene.

More specifically, the present invention provides a method for enhancing root
15   growth of plants or enhancing the resistance in plants to the obstacle-touching stress by
overexpressing the *GmEXP1* gene in the plants.

For overexpression, the *GmEXP1* gene is introduced into plants with or without
the *GmEXP1* gene.   The "overexpression" means the expression of the *GmEXP1* gene
20   at a level higher than that in wild-type plants.   As a method for introducing a gene into
a plant, there is a method for transforming a plant using an expression vector including
the gene controlled by a promoter.   Any promoter can be used if it can overexpress the
gene introduced into the plant.   The promoter may be, but not limited to, 35S RNA or
19S RNA promoter of CaMV, a full-length transcription promoter derived from Figwort

mosaic virus (FMV), or a TMV coat protein promoter. Also, an ubiquitin promoter

can be used to overexpress the gene in a monocotyledon or a woody plant.

The present invention is applicable to both monocotyledons and dicotyledons.

5   The monocotyledons include, but not limited to, rice, wheat, barley, bamboo shoot, corn,

taro, asparagus, onion, garlic, shallot, leek, yam and ginger.   The dicotyledons include,

but not limited to, Arabidopsis, eggplant, tobacco, cayenne, tomato, burdock, crown

daisy, lettuce, Chinese bellflower, spinach, red beet, sweet potato, celery, carrot, parsley,

Chinese cabbage, cabbage, leaf radish, watermelon, melon, cucumber, pumpkin, gourd,

10   strawberry, soybean, mung bean, kidney bean and pea.

The *GmEXP*1 polypeptide and polynucleotide encoding the polypeptide of the

present invention can be utilized in the genetic improvement for root growth of plants

and in the investigation of root growth regulating genes in other plants by known

15   genetic engineering techniques, such as DNA chip, protein chip, polymerase chain

reaction, Northern blot analysis, Southern blot analysis, enzyme-Linked Immunosorbent

assay and 2-D gel analysis.

Also, the present invention provides a method for identifying materials

20   influencing the activity of the polypeptide or the expression of the gene according to the

present invention.   More specifically, the present invention provides a method for

identifying a compound influencing the activity of the polypeptide or the expression of

the gene provided by the present invention, comprising the steps of contacting the

polypeptide or a recombinant cell for expression of the polypeptide and a candidate

material and measuring an effect on the activity of the polypeptide or the expression of

the gene.   The effect on activity or expression means enhancement of root growth and

improvement of the resistance to the obstacle-touching stress by enhancing the activity

of the polypeptide or the expression of the gene.   The effect of a candidate material on

5   the polypeptide or gene of the present invention can be evaluated by a known method

such as Northern blot or Southern blot analysis.   Compounds, which may affect the

activity of the polypeptide or the expression of the gene of the present invention,

include peptides, polypeptides, peptide copies, compounds and biologicals.

10   **Best Mode for Carrying out the Invention**

Hereinafter, the present invention will be described in detail by examples.

It is to be understood, however, that these examples are for illustrative purpose

only and are not construed to limit the scope of the present invention.

15

<Example 1>

Cloning of Expansin Genes in Soybean (*Glycine max* cv *Paldal*) roots

To isolate expansin cDNAs from soybean, primers targeted to the conserved

20   regions of known expansin genes were designed, and PCR was carried out with soybean

genomic DNA as a template.   The conserved common regions of the expansin genes

CsEXP1 (GenBank Accession No. U30382) and *CsEXP2* (GenBank Accession No.

U30460) of cucumber (*Cucumis*), the expansin gene *AtEXP5* (GenBank Accession No.

U30478) of *Arabidopsis*, the expansin genes *OsEXP2* (GenBank Accession No.

U30477) and *OsEXP3* (GenBank Accession No. U30479) of rice (*Oryza*) and the

expansin gene *LeEXP1* (GenBank Accession No. U82123) of tomato (*Lycopersicon*)

were analyzed using the website address at http://ncbi.nlm.nih.gov/Entrez/ and Clustal

V software.   To synthesize the conserved common regions of the above genes by PCR

5   (polymerase chain reaction), two degenerate (forward and reverse) primers as

mentioned below were synthesized.

Forward primer (SEQ ID No. 3)

5'-NNGGATCCGAYGCNTCNGGNACNATGGGYGGYGCTGYGYTAN GG-

10   3'

Reverse primer (SEQ ID No. 4)

5'-NNGGATCCTTKSWYTGCCARTTNNSNCCCCARTTNCK-3'

Wherein Y is T or C, K is T or G, S is C or G, and R is A or G.

15   PCR was carried out with soybean genomic cDNA as a template using the above

primers.   For 50 μl of a final reacting solution obtained by mixing 1 x PCR buffer, 200

μM of each dNTP, 1 μM of each primer, 1.5 mM MgCl$_2$ and 2.5 units of *Taq* DNA

polymerase (Bioneer, Daejon, Korea), PCR was performed at 94°C for one minute, at

60°C for one minute and at 72°C for two minutes.   The same reaction was repeated 35

20   times.

The amplified PCR product was isolated from a gel and purified by Geneclean II

kit (BIO 101), and its nucleotide sequence was determined by the Sanger method (the

dideoxynucleotide chain termination method)(Sanger F. T. et al., *Proc. Natl. Acad. Sci*

USA, 74:5463, 1997).   The amplified DNA fragments were labeled using the Prime-a-

Gene system (Promega, Madison, WI) and used as a probe (SEQ ID No. 5) for

screening expansin genes in the Uni-ZAP XR vector (Stratagene, La Jolla, CA) that is a

cDNA library of soybean roots.

5   After screening the Uni-ZAP XR vector (Stratagene, La Jolla, CA) (Sambrook J.

et al., *Molecular Cloning; A Laboratory Manual*, Ed. 2, Cold Spring Harbor Laboratory

Press, Cold Spring Harbor, NY, 1989), one positive clone was isolated.

<Example 2>

Determination of the Nucleotide sequence of cDNA of Expansin Genes

10   **Isolated from Soybean Roots and Evaluation of Sequence Homology**

         The nucleotide sequence of the clone isolated in Example 1 was determined by a

kit (Sequence version 2.0 kit, USB) using the Sanger method.   The determined DNA

nucleotide sequence was analyzed using a sequence analysis program (Mac DNASIS

15   program, Hitachi Software Engineering, America Ltd., San Bruno, CA), and an amino

acid sequence was analogized from the base sequence.   Also, the sequence homology

between the nucleotide sequence of the expansin gene of the present invention and that

of other known expansin genes was analyzed.

         As a result, it was revealed that the isolated cDNA consists of 1,089bp with an

20   open reading frame encoding a polypeptide of 255 amino acids, which contains a

putative signal sequence of 16 amino acids at the N terminus.   Also, the homology

analysis showed that the gene of the present invention has strong sequence similarities

to a group of α-expansins including *NtEXP3 of* tobacco (86%; Link B.M. et al., *Plant*

Physiol., 118:907-916, 1998), *CsEXP2* of cucumber (92%; Shcherban T.Y. et al., *Proc. Natl. Acad. Sci.*, USA, 92:9245-9249, 1995), *OsEXP1* of rice (85%; Cho H-T t al., *Plant Cell*, 9:1661-1671, 1997b) and *AtEXP1* of Arabidopsis (82%; Shcherban T.Y. et al., *Proc. Natl. Acad. Sci.*, USA, 92:9245-9249, 1995) (See FIG. 1). Thus, the inventors

5   termed the new expansin gene isolated from the soybean root "*GmEXP1*". The nucleotide sequence and amino acid sequence of the *GmEXP1* gene are shown in SEQ ID No. 1 and SEQ ID No. 2. Also, the nucleotide sequence of the inventive *GmEXP*1 gene was registered for GenBank(GenBank Accession No. AF516879).

10   **<Example 3>**

Phylogenetic Classification of *GmEXP1*__

The *GmEXP1* gene having high-level homology to the α-expansin genes was classified by a phylogenetic analysis. The phylogenetic tree for 20 known α-expansin

15   genes and the expansin gene of the present invention was constructed by the maximum likelihood method (HKY 85 model) using PAUP program. Known α-expansin genes as used in this example are *AtEXP6* (GenBank Accession No. U30480), *AtEXP1* (GenBank Accession No. U30476), *AtEXP* (GenBank Accession No. U30478) and *AtEXP2* (GenBank Accession No. U30481) derived from Arabidopsis (*Arabidopsis*

20   *thaliana*); *PsEXP1* (GenBank Accession No. 85187) derived from pea (*Pisum sativum*); *LeEXP1* (GenBank Accession No. U82123), *LeEXP18* (GenBank Accession No. AJ004997) and *LeEXP2* (GenBank Accession No. AF096776) derived from tomato (*Lycopersicon esculentum*); *NtEXP4* (GenBank Accession No. AF049353), *NtEXP5* (GenBank Accession No. AF049354), *NtEXP1* (GenBank Accession No. AF049350),

NtEXP2 (GenBank Accession No. AF49351) and *NtEXP3* (GenBank Accession No.

AF049352) derived from tobacco (*Nicotiana tabacum*); *PtEXP1* (GenBank Accession

No. U64892) derived from loblolly pine (*Pinus taeda*); *CsEXP2* (GenBank Accession

No. U30477) derived from cucumber (*Cucumis sativus*); *OsEXP1* (GenBank Accession

5   No. Y07782), *OsEXP 2* (GenBank Accession No. U30477), *OsEXP3* (GenBank

Accession No. U30479) and *OsEXP4* (GenBank Accession No. U85246) derived from

rice (*Oryza sativa*); and *PHLP1* (GenBank Accession No. X78813) derived from

timothygrass (*Phleum pratense*).

The analysis results show that the *GmEXP1* gene of the present invention

10   belongs to Group D of the α-expansin subfamily (See FIG. 2).

<Example 4>

Analysis of *GmEXP1* Gene Present in Soybean Genome

15   To estimate the presence and number of the *GmEXP1* gene in the soybean

genome, a Southern blot analysis was performed with part of the *GmEXP1* gene as a

probe.   A genomic DNA was isolated from leaves of soybean grown in a Hoagland

solution for 20 days under long-day condition (16-hour light/8-hour dark) at 26°C,

utilizing DNeasy Plant Mini Kit (QIAGENE®).   10 μg of the extracted DNA was

20   digested with different restriction enzymes *Eco*RV, *Hin*dIII and *Nco*I.   The digested

DNA is separated in a 1% agarose gel and transferred onto a Hybond N^{+} nylon

membrane (Amersham Biosciences AB, Uppsala).   A Southern blot analysis was

performed with the DNA fragments (nucleic acid Nos. 52 to 816 of *GmEXP1*)

containing the coding region of *GmEXP1* or the DNA fragments (nucleic acid Nos. 603

to 991 of *GmEXP1*) containing the coding region and the 3'-untranslated region of

GmEXP1 as a probe.   The probe was labeled with the radioactive isotope $a^{-32}P$ and

hybridized with the nylon membrane, to which the genomic DNA is attached, at 65°C

(Ahn J.H. et al., *Plant Physiol.*, 116:671-679, 1998).

5   As a result, in the case of the hybridization with only the coding region of the

GmEXP1 cDNA, many bands were detected in each lane (See FIG. 3A), implying that

various expansin genes with homology to the α-expansins are present in the soybean

genome.   However, when a probe specific only for the *GmEXP1* gene including both

the coding region and the 3'-untranslated region was used in hybridization, a strong

10   single band appeared along with occasional weaker bands in each blot (See FIG. 3B).

It suggests that the *GmEXP1* gene exists as a single-copy gene in the soybean genome.

          <Example 5>

          Expression Patterns of *GmEXP1* in Different Tissues of Soybean

15

          The expression patterns of the *GmEXP1* gene were investigated in different

tissues of soybean.   Total RNAs were isolated from the leaves, roots, stems and

hypocotyls of soybean seedlings grown for 20 days under the same condition as used in

Example 4.   Northern blot analysis was performed with a *GmEXP1*-specific probe.

20   RNeasy Plant Kit (QIAGENE®) was used to isolate the RNAs from different tissues.

10 μg of the isolated RNA was separated in a 1% agarose gel containing formaldehyde,

transferred onto a nylon membrane, and hybridized with the probe at 65°C.

          As a result, hybridization signals were detected only in the RNA isolated from

roots (See FIG. 4), indicating that the *GmEXP1* gene is root-specific and may be involved in root development.   The size of the *GmEXP1* mRNA was about 1.1 kb, which is similar to that predicted from its cDNA sequence.

5   **<Example 6>**

           Expression Patterns of *GmEXP1* in Soybean Roots at Different Developmental Stages

           The expression patterns of the *GmEXP1* gene were evaluated in soybean roots

10   at different developmental stages by the same method as used in Example 5.   Total RNAs were extracted from roots of 1-, 2-, 3-, 4-, 5-, 7-, 9- and 13-day old soybean seedlings.   Northern blotting was performed with a *GmEXP1*-specific probe by the same method as used in Example 5.

           The growth pattern of soybean roots was compared with the expression pattern

15   of the *GmEXP1* gene.   The growth pattern of soybean roots was monitored by measuring the root lengths of the primary, secondary and tertiary roots of six young seedlings grown in a liquid medium.   The expression pattern of the *GmEXP1* gene was detected by isolating RNAs from the primary and secondary roots 5 days after germination, using Northern blot analysis.

20   As a result, the *GmEXP1* gene was expressed in all root developmental stages. Particularly, the expression level was very high 1 day after germination and reached the maximum level 5 days after germination (See FIG. 5).

           The primary roots grew rapidly until 4 days after germination, and then their growth rate was gradually decelerated.   The secondary roots were initiated from the

primary root 2 days after germination and grew rapidly from 4 to 6 days after

germination.   The tertiary roots emerged 8 days after germination (See FIG. 6).

Although the *GmEXP1* gene was expressed in both the primary roots and the secondary

roots of 5-day old seedlings, the expression level was much higher in the secondary

5   roots (See FIG. 7).   This is probably because the growth of the primary roots was

decelerated from 5th day of germination, whereas the growth of the secondary roots was

accelerated.   Furthermore, the expression levels of the *GmEXP1* gene were gradually

decreased as the growth of the secondary roots was decelerated.   Therefore, it suggests

that the *GmEXP1* gene is involved in root development and that the expression of the

10   *GmEXP1* gene is up-regulated when rapid root elongation takes place during root

development in soybean.

<Example 7>

Spatial Expression Patterns of *GmEXP1* in Soybean Roots

15

The spatial expression patterns of the *GmEXP1* gene were analyzed by

Northern blot and in situ hybridization.   That is, the expressions of the *GmEXP1* gene

in the 5-day-old primary roots and 9-day-old seedlings that showed the maximum level

of *GmEXP1* expression were determined.

20

<7-1> Northern Blot

The primary roots were serially dissected into 8 sections (FIG. 8A), and the

secondary roots were serially dissected into 5 sections (FIG. 8C).   Total RNAs were

isolated from each section and subjected to a Northern blot analysis by the same method

as used in Example 5.

As a result, the *GmEXP1* transcripts were predominantly detected in section 1

5   of the primary roots, representing the root tip region of cell division and elongation

(Ahn J.H. et al., *Plant Physiol.*, 116:671-679, 1998).   In contrast, the expression level

was very low in sections 2 to 8 (See FIG. 8B).   Interestingly, a slightly elevated level of

the *GmEXP1* expression was detected in section 6, which was most likely contributed

by the emerging secondary roots.   Even in the secondary roots, the expression level

10   was high in section 1 that includes the tip region (See FIG. 8D).   Therefore, it is

concluded that the *GmEXP1* gene is highly expressed in a specific zone that includes the

region of cell division and elongation in the primary and secondary roots, and that the

GmEXP1 gene plays an important role in cell elongation during root development.

15   **<7-2> In Situ Hybridization**

In situ hybridization was used to detect the regions where the *GmEXP1* gene is

expressed.   The hybridization was performed using the primary roots of 5-day old

soybean seedlings by modifying a known method (Glik B.R. et al., *Methods in Plant*

20   *Molecular Biology and Biotechnology*, CRC Press, 179-205, 1993; Cho H-T et al.,

Plant J., 15:805-812, 1998).   The primary roots of the soybean seedlings were

immediately immersed in the formaldehyde-acetic acid-fixation solution containing

50% (v/v) ethanol, 5% (v/v) acetic acid and 3.7% (v/v) formaldehyde, and the tissues

were subsequently fixed and embedded in paraplast.   The embedded root tissues were

vertically or horizontally sliced into 8-μm thick sections using a microtome (Leica

Instruments GmbH, Wetzlar, Germany).   When the roots were horizontally cut,

sections were selectively obtained from the regions of cell division, elongation and

maturation based on the anatomical features.   For the in situ hybridization, a probe

5   synthesizing a DIG-labeled RNA probe (antisense: SEQ ID No. 6 or sense: SEQ ID No.

7) with the *GmEXP1*-specific DNA fragments in Example 4 as a template was used.

That is, the *GmEXP1*-specific DNA fragments were digested with *Eco*RV, *Hin*dIII and

*Nco*I and inserted into the pSTP18 vector (Roche Diagnostics, Manheim, Germany).

The RNAs were obtained using SP6 or T7 polymerase with the plasmid as a template.

10   The RNAs are in the form of a mixture of digoxigenin and UTP.   The sense probe was

used as the control group.   The synthesized DIG-labeled RNA probe was hybridized

with the soybean root tissue fragments at 65°C, and reacted with 1st DIG antibody

(Boehringer Manheim) which specifically reacts to digoxigenin labeled with alkaline

phosphatase.   Upon completion of the reaction, the resulting product was dyed with

15   nitroblue tetrazolium/5-bromo-4-chloro-3-indolylphosphate and observed using a

microscope (Optiphot-2, Nikon, Tokyo).

        The results of in situ hybridization were identical to those obtained by RNA

genome gel blot in Example <7-1>.   The *GmEXP1* gene was highly expressed in the

root tip region of the primary roots of soybean seedlings.   When longitudinal sections

20   of the primary roots were hybridized with an antisense probe, the expression levels of

the *GmEXP1* gene gradually increased from the region of cell division to the region of

elongation and then decreased in the region of maturation (See FIG. 9A).   In case of

hybridization with a sense RNA probe, no expression of the *GmEXP1* gene was

detected (See FIG. 9B).   The elongation region of the primary root, which was

27

hybridized with the antisense RNA probe, was observed upon magnification.   It was

detected that the *GmEXP1* gene was highly expressed in the epidermis, some underlying

cell layers and the vascular cylinder in the region of elongation (See FIG. 9C).   Also, in

the cross sections of the primary roots of the soybean seedlings, the *GmEXP1* gene

5   showed low expression level at the cells in the root cap (See FIG. 10A) and a high

expression level at cells of epidermis and vascular cylinder in the region of elongation

(See FIGs. 10B and 10C).   However, the *GmEXP1* gene showed low-level expression

at the epidermal cells in the maturation region (See FIG. 10D).   These results imply

that the *GmEXP1* gene is expressed in the regions of cell division and elongation,

10   preferentially in their epidermal cells and some underlying cell layers.

\<Example 8\>

Relationship Between *GmEXP1* Expression and Root Initiation

15   To test whether *GmEXP1* gene is involved in formation of the root initials, the

expression patterns of the *GmEXP1* gene were investigated in the secondary root initials.

The secondary root-initiating region in the primary root of 5-day old soybean seedlings

(about 3.5 cm from the root tip) was longitudinally or horizontally sectioned and

subjected to in situ hybridization analysis.   In situ hybridization was performed using a

20   sense RNA probe or an antisense RNA probe in the same manner as described in

Example \<7-2\>.

As a result, it was detected that the *GmEXP1* gene was expressed in the

secondary root initials, particularly in their epidermal cells (See FIG. 11A).   In the

cross sections of the primary root hybridized with an antisense RNA probe, the

GmEXP1 gene was mainly expressed in the tip region of the emerging secondary root

(See FIG. 11B).   In contrast, the sections hybridized with a sense probe did not show

any detectable expression of the *GmEXP1* gene (See FIG. 11C).   It is known that the

secondary root is initiated from the pericycle in the region of maturation of the primary

5   root and then penetrates through the cell layers of cortex and epidermis to emerge from

the primary root (Malamy J.E. et al., *Development*, 124:33-44, 1997).   Although the

origin of the secondary root is different from that of the primary root, they commonly

require the *GmEXP1* gene for cell elongation and have no significant difference in their

morphology, organization or gene expression (Dolan L. et al., *Development*, 119:71-84,

10   1993).

<Example 9>

Reaction of Tobacco Transformed with *GmEXP1* in Acidic Condition and Insensitivity to Obstacle-Touching Stress

15

<9-1> Transformation of Tobacco

The *GmEXP1* gene was overexpressed in tobacco plants in order to identify the

function of the gene in plants.   For amplification of the coding region (765bp) of the

20   *GmEXP1* cDNA, a pair of primers as mentioned below was synthesized by a known

method.

Forward primer (SEQ ID No. 8)

5'-ACCAAGCTTCAACCTCTCATCATTAGGC-3'

Reverse primer (SEQ ID No. 9)

5'-ACCAAGCTTGGAGTTGATGGGAATAATCA-3'

5   PCR was carried out with soybean cDNA library (Uni-ZAP XR vector) as a
template using the above primers.   The PCR-amplified product was digested with
*Hin*dIII and then inserted into the *Hin*dIII site of the pGA643 vector containing the
CaMV 35S promoter and *NOS* terminator (An G. et al., *Binary Vectors*, Plant Molecular
Biology Manual, Kluwer Academic Publishers, A31-A319).   The vector was
introduced into *Agrobacterium tumefaciens* cells by electroporation. *A. tumefaciens*-
10   mediated tobacco plants transformation was performed as a known method (Holsters M.
et al., *Mol. Gen. Genet.*, 163:181-187, 1978; Horsch R.B. et al., *Science*, 227:1229-1231,
1985).   The transformed (transgenic) tobacco plants were cultured and selected on
Murashige and Skoog basal medium supplemented with 200 mg/L of kanamycin and
500 mg/L of carbenicillin.

15

<9-2> Change in the Phenotype of Transgenic Tobacco

The phenotype of the transgenic tobacco plants obtained in Example <9-1> was
compared with that of the wild-type tobacco plants.   Also, the transgenic plants with
20   overexpressed *GmEXP1* gene were dissected in the same manner as described in
Example <7-2> and the anatomical features of the transgenic plants were observed by a
microscope.

As a result, the transgenic plants with overexpressed *GmEXP1* gene were
relatively bigger than the wild-type plants.   This suggests that the overexpression of

the *GmEXP1* gene accelerates the growth of plants.   In addition, abnormal phenotypes were detected in the leaves, stems and flowers of the transgenic plants.   The strong lines at which the *GmEXP1* gene was highly expressed showed a bushy phenotype, because multiple leaves developed simultaneously from the shoot apex.   In contrast,

5   the weak lines at which the *GmEXP1* gene showed low-level expression showed a phenotype generating auxiliary shoots, probably due to weak apical dominance (data not shown).   In terms of anatomical features, the cells in the leaves of the strong lines were enlarged and layered irregularly (FIGs. 12A and 12B).   Also, the transgenic plants showed thickened xylem cell layers in the stems (FIGs. 12C and 12D), although there

10   was no significant difference in xylem cells of the petioles of the transgenic plants as compared to those of the wild-type plants (FIGs. 12E and 12F).   It was also detected that the root tip region of the transgenic plants was more elongated than that of the wild-type plants(FIG. 13).   The analysis results indicate that the overexpression of the *GmEXP1* gene accelerates the growth of plants and selectively affects developmental

15   processes of transgenic plants.

<9-3> Growth Rate of Transgenic Plants in Acidic Condition and Sensitivity to Obstacle-Touching Stress

20   In order to observe the growth rate of transgenic tobacco roots under acidic condition, the transgenic tobacco plants with low expression level of the *GmEXP1* gene were self-pollinated to obtain homozygous seeds in the T_2 generation, because transgenic plants with a high expression level of the *GmEXP1* gene are sterile.   The T_2 seeds were sterilized with 10% (w/v) bleach solution for 10 minutes and washed several

times with sterile water.   The seeds were sown on 1.5% (w/v) agar plates containing

0.5 x Murashige and Skoog basal medium (Invitrogen, Carlsbad, CA) and which are

adjusted to pH 4.5 or 7.0.   As a control group, wild-type soybean seeds were sown on

the agar plates in the same manner.   The homozygous transgenic seeds and the wild-

5   type seeds were incubated on the plates vernalized by cold treatment (for 2 days in the

dark) in a vertical position under long-day condition (16-hour light/8-hour dark) at 26°C.

The lengths of their primary roots were measured 17 days after germination.

         As a result, it was detected that the primary roots of the transgenic seedlings are

longer than those of the wild-type seedlings under both neutral and acidic conditions.

10   Particularly, transgenic seedlings grown under acidic condition had longer root length

than those grown under neural condition (See FIG. 14).   It is known that the acidic

condition accelerates the growth of plant tissues.   The transgenic seedlings with

overexpressed *GmEXP1* gene appear to have been more reactive in the acidic condition,

thereby having longer roots than the wild-type seedlings.   These results suggest that

15   the *GmEXP1* gene induces acid growth in soybean.

         Also, in order to investigate the resistance (insensitivity) of the transgenic

tobacco roots to the obstacle-touching stress, the T_2 seeds and the wild-type seeds were

sterilized and incubated on pH 4.5 agar plates containing the MS medium in the same

manner as described above.   After vernalization (cold treatment) for 2 days, the plates

20   were erected in a vertical position(90°).   On the 3rd day, one half of the plates were

inclined at 45° to give the obstacle-touching stress, and the rest were continually

incubated in a vertical position.   The seeds were incubated under long-day condition

(16-hour light/8-hour dark) at 26°C.   The lengths of their primary roots were measured

17 days after germination.

As a result, in the vertical plates, the primary roots of the wild-type soybean seedlings, which did not encounter the obstacle-touching stress, just grew straight downward on the surface of the agar.   In contrast, the primary roots of the wild-type seedlings, which were affected by the obstacle-touching stress, were much shorter.

5   The average root length was 3.5 cm under no obstacle-touching stress.   The root length was reduced to 1.1 cm under the obstacle-touching stress.   However, the primary roots of *GmEXP1* overexpressing seedlings were barely affected by the obstacle-touching stress and much longer than those of the wild-type seedlings (See FIG. 15).   From these results, it is clear that the *GmEXP1* overexpression improves the resistance to the

10   obstacle-touching stress, and that the *GmEXP1* gene plays an important role in overcoming the stress, which the primary roots would encounter in soil.

<9-4> Length of Root Epidermal Cells in Transgenic Seedlings

15   The same manner described in Example <9-3>, wild-type and homozygous transgenic seeds were incubated on 1.5% (w/v) agar plates (pH 4.5) in an inclined position at 45° to encounter the obstacle-touching stress.   The seeds were incubated under long-day condition (16-hour light/8-hour dark) at 26°C.   The lengths of the epidermal cells in their roots and stems were measured 17 days after germination.   The

20   cell length was measured using the NIH image software (developed by the United States National   Institute   of   Health   and   available   on   the   Internet   at http://rsb.info.nih.gov/nihimage).

As shown in Table 1, when compared to the roots of the wild-type seedlings, the roots of the transgenic seedlings overexpressing the *GmEXP1* gene showed significant

elongation of epidermal cells, which is likely to be a main reason for the rapid growth.

However, there was no significant difference in the epidermal cell length in stems and

vascular cylinder between the transgenic roots and the wild-type roots (data not shown)

5   Table 1. Comparison of Length of Root Epidermal Cells

Distance from	Cell Length	
Root Tip	Wild Type Soybean	Transgenic Soybean
400	17.6 ± 3.9	30.2 ± 6.9
800	32.0 ± 5.0	71.9 ± 15.7
1,200	140.5 ± 21.6	216.0 ± 25.5
1,600	188.7 ± 33.7	207.9 ± 30.7

Effect of the Invention

The *GmEXP1* gene of the present invention and the expansin protein expressed from the *GmEXP1* gene can be utilized in the characteristic improvement for root growth of plants and in the investigation of root growth regulating genes in other plants.

5   Also, the *GmEXP1* gene plays important roles in enhancing root growth of plants and enhancing resistance in plants to obstacle-touching stress.

WHAT IS CLAIMED IS:

1. A protein controlling root growth of plants, comprising an amino acid sequence represented by SEQ ID NO. 2

5

2. The protein according to claim 1, which is isolated from soybean(*Glycine max*).

3. A gene encoding the protein of claim 1.

10

4. The gene according to claim 3, having a root-specific expression pattern.

5. The gene according to claim 3 or 4, which is a *GmEXP*1 gene having a nucleic acid sequence represented by SEQ ID NO. 1

15

6. A recombinant vector comprising the gene of claim 3.

7. The recombinant vector according to claim 6, which is pGA643/*GmEXP*1 comprising the gene of claim 5.

20

8. Bacteria transformed with the recombinant vector of claim 6.

9. A method for enhancing root growth of a plant, comprising the steps of inserting the gene of claim 3 into a plant expression vector and introducing the inserted

gene into the plant.

10. A method for enhancing resistance in a plant to obstacle-touching stress, comprising the steps of insertion the gene of claim 3 into a plant expression vector and introducing the inserted gene into the plant.

11. The method according to claim 9 or 10, wherein the plant is a monocotyledon or a dicotyledon.

12. The method according to claim 11, wherein the monocotyledon is selected from the group consisting of rice, wheat, barley, bamboo shoot, corn, taro, asparagus, onion, garlic, shallot, leek, wild rocambole, yam and ginger.

13. The method according to claim 11, wherein the dicotyledon is selected from the group consisting of Arabidopsis, eggplant, tobacco, cayenne, tomato, burdock, crown daisy, lettuce, Chineses bellflower, spinach, red beet, sweet potato, celery, carrot, dropwort, parsley, Chineses cabbage, cabbage, leaf radish, watermelon, melon, cucumber, pumpkin, gourd, strawberry, soybean, mung bean, kidney bean and pea.

14. A method for identifying a compound affecting the activity or expression of the above protein, comprising the steps of culturing the protein of claim 1 or a recombinant cell expressing the protein of claim 1 and a candidate material and measuring an effect on the activity or expression of the protein.

15. The method according to claim 14, wherein the compound enhances the activity or expression of the protein of claim 1.

Fig. 1

```
GmEXP1    MGK~IMLVLGSLIGLCCFTIT--TYA-------FSPSGWTNAHATFYGGSD
NtEXP3    MAKCGILALGFIIGF--FSIFFNANA-------FTASGWMRAHATFYGGAD
CsEXP2    MEK-LPFAFAFFLALSNFFFLF-VNA-------FTASGWAPAHATFYGESD
AtEXP6    MA------MLGLVLSVSVLTTILALSEARIPGVYNGGGWETAHATFYGGSD
PsEXP1    MA------AI~LLLTIASLTSLFAPTTARIPGVYTGGPWTSAHATFYGGSD
          *          .    .       .              .  . *  ******* .*

GmEXP1    ASGTMGGACGYGNLYATGYGTRTAALSTALFNDGASCGQCYKIICDYKSD
NtEXP3    ASGTMGGACGYGNLYSTGYGTRSAALSTALFNSGGSCGQCYKIICDFYAE
CsEXP2    ASGTMGGACGYGNLYQTGYGTRTAALSTALFNDGASCGQCFKIICDYKTD
AtEXP6    ASGTMGGACGYGNLYSQGYGVNTAALSTALFNNGFSCGACFELKCA--SD
PsEXP1    ASGTMGGACGYGNLYSQGYGVNTAALSTALFNNGLSCGACFELKCD--QD
          ******* *******   ***  .********* * *** *. . *      .

GmEXP1    SRWCIKGR~SVTVTATNFCPPNFALPNNNGGWCNPPLKHFDMAQPAWEKI
NtEXP3    PRWCKKGV-SVTITATNFCPPNYALPSDNGGWCNPPRQHFDMAQPAWEKI
CsEXP2    PRWCIKGA-SVTITATNFCPPNYALPNNNGGWCNPPLKHFDMAQPAWQKI
AtEXP6    PKWCHSGSPSIFITATNFCPPNFAQPSDNGGWCNPPRPHFDLAMPMFLKI
PsEXP1    PRWCNPGNPSILITATNFCPPNFAEPSDNGGWCNPPRPHFDLAMPMFLKI
          .. **   *  *. .*********. * *.. ********  ***.* *     **

GmEXP1    GIYRGGIVPVLFQRVPCKKHGGVRFSVNGRDYFELVLISNVGGAGSIQSV
NtEXP3    GVYRGGIIPVFYQRVPCKKRGGVRFTINGRDYFELVLVSNVGGAGSVRSV
CsEXP2    GIYRGGIIPVLYQRVPCKKRGGVRFTVNGRDYFELVLITNVGGAGDIKSV
AtEXP6    AEYRAGIVPVSFRRVPCRKRGGIRFTINGFRYFNLVLVTNVAGAGNIVRL
PsEXP1    AQYRAGIVPVAYRRVPCRKAGGIRFTINGFRYFNLVLITNVAGAGDIVRV
          .  **.**.**  ..****.* **. * .**    *.***..**.***

GmEXP1    FIKGSKTG~WMAMSRNWGSNWQSNAYLNGQSLSFRVTTTDGETRVFQDIV
NtEXP3    QIKGSRT~NWMTMSNNWGANFQSNTYLNGQSLSFRVTTTDGVTKTFLNIV
CsEXP2    SIKGSKSSNWTPMSRNWGANWQSNSYLNGQSLSFKVTTSDGQVQVFNMVV
AtEXP6    GVKGTHTS~WMTMSRNWGQNWQSNSVLVGQSLSFRVTSSDRRSSTSWNIA
PsEXP1    SVKGTNTA~WMTMSRNWGQNWQSNAVFVGQALSFRVTGSDRRTSTSWNVA
          *. .  *   ** *** * ***. . **.***.** .*           ..

GmEXP1    PVSWTFGQTFSSPVQF-
NtEXP3    PANWRFGQTFSSPTQFS
CsEXP2    PSSWRFGQTFASKVQFS
AtEXP6    PANWKFGQTFMGKNFRV
PsEXP1    PPHWQFGQTFTGKNFRV
          *   * *****  .
```
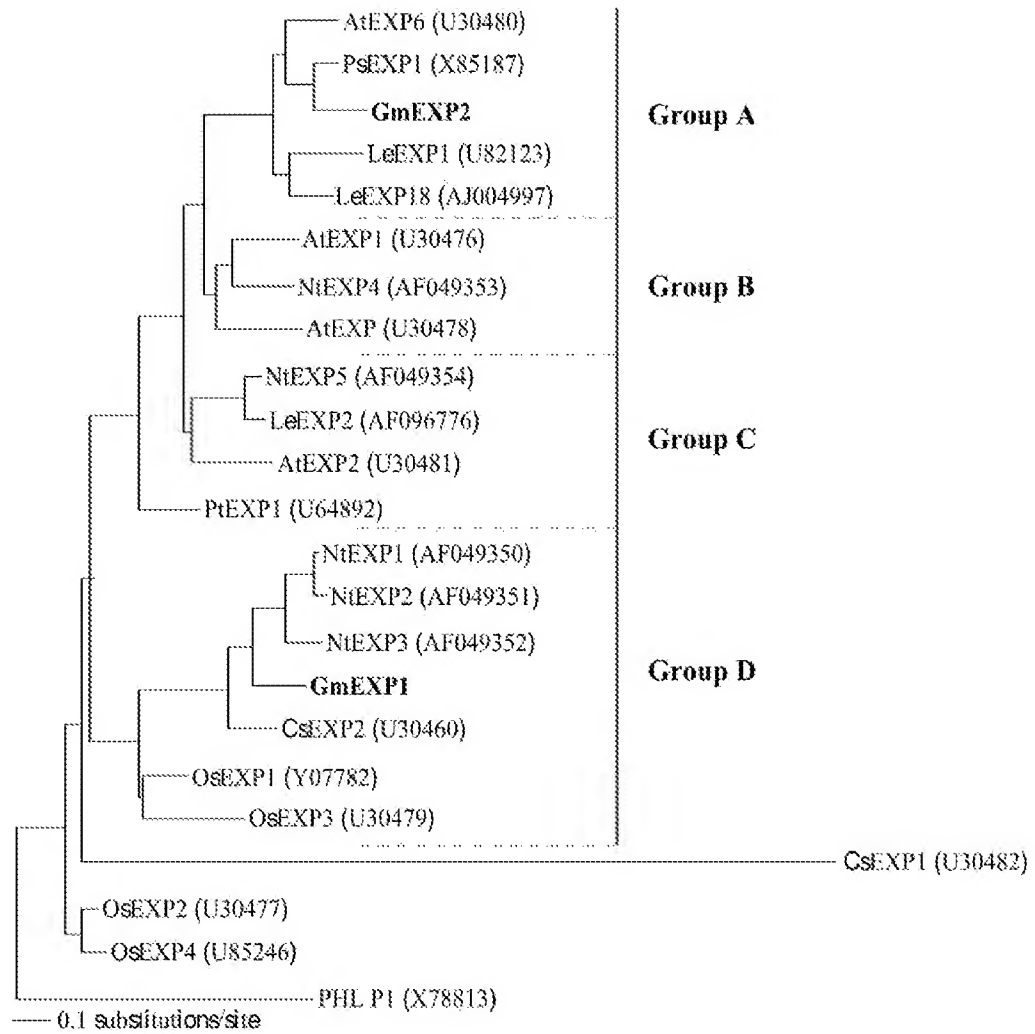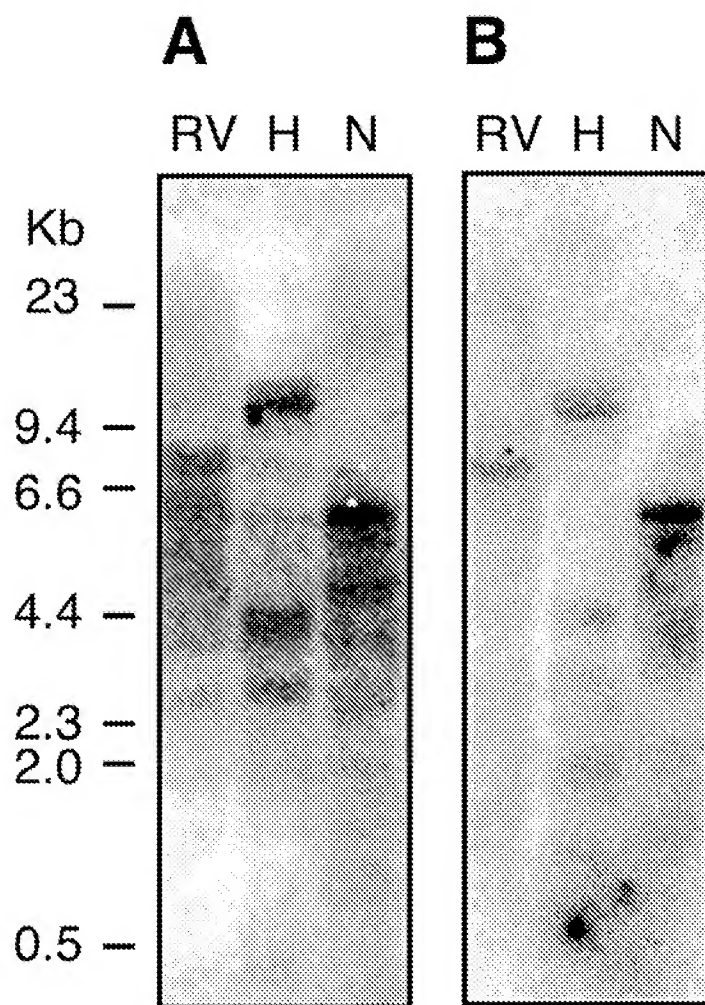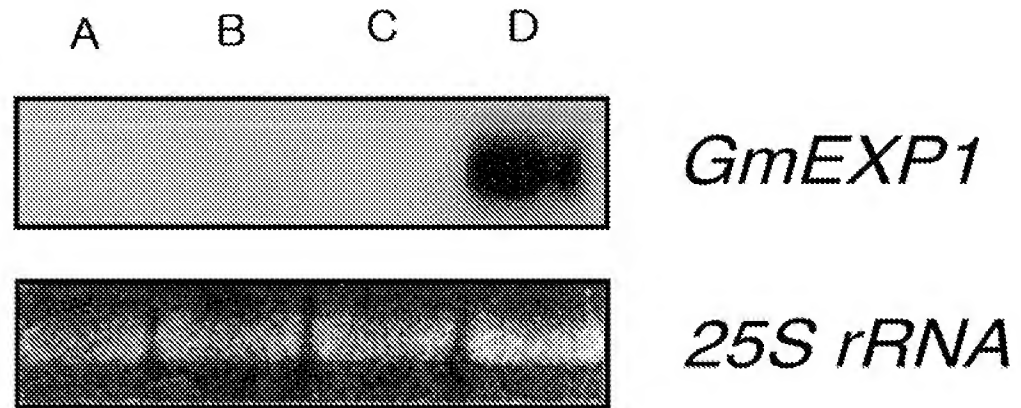
Fig. 2

AtEXP6 (U30480)
PsEXP1 (X85187)
GmEXP2
LeEXP1 (U82123)
LeEXP18 (AJ004997)
Group A

AtEXP1 (U30476)
NtEXP4 (AF049353)
AtEXP (U30478)
Group B

NtEXP5 (AF049354)
LeEXP2 (AF096776)
AtEXP2 (U30481)
Group C

PtEXP1 (U64892)

NtEXP1 (AF049350)
NtEXP2 (AF049351)
NtEXP3 (AF049352)
GmEXP1
CsEXP2 (U30460)
Group D

OsEXP1 (Y07782)
OsEXP3 (U30479)

CsEXP1 (U30482)

OsEXP2 (U30477)
OsEXP4 (U85246)

PHL P1 (X78813)

0.1 substitutions/site

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

A

B

GmEXP1

25S rRNA

C

D

GmEXP1

25S rRNA
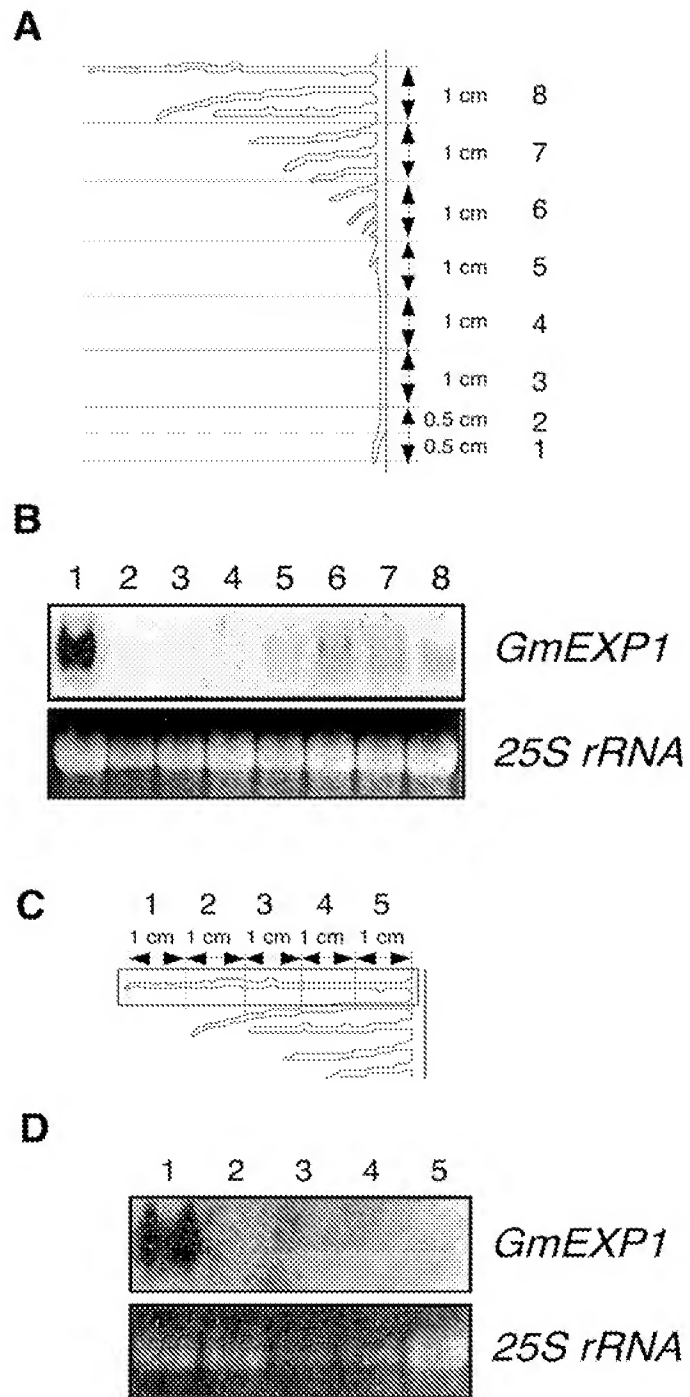
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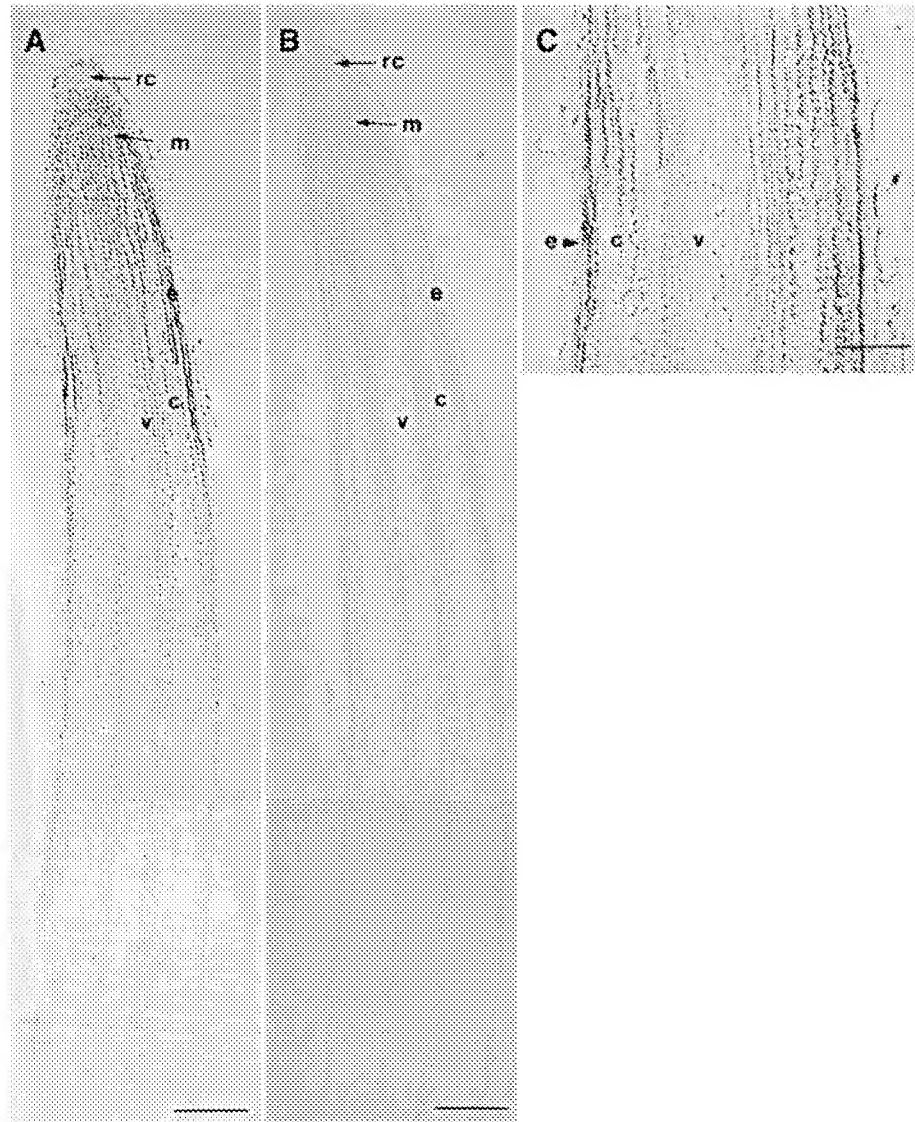
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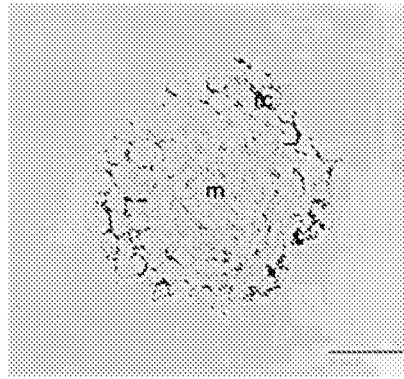
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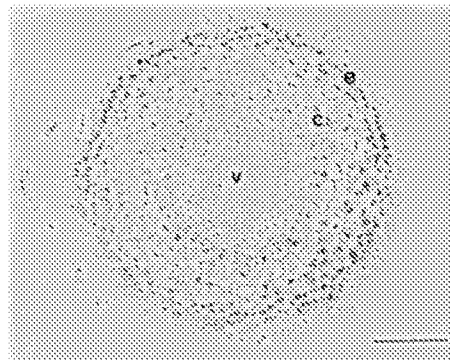
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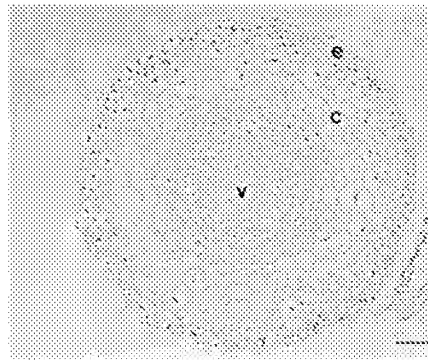
D

Fig. 11

Fig. 12

Fig. 13

Fig. 14

Fig. 15

<110>   Seoul National University Industry Foundation

<120>   Gene controlling root development of plants and method for manipulating root development of plant using the same

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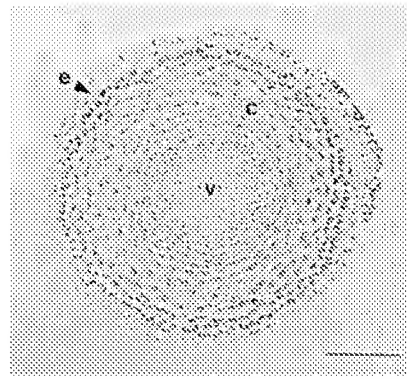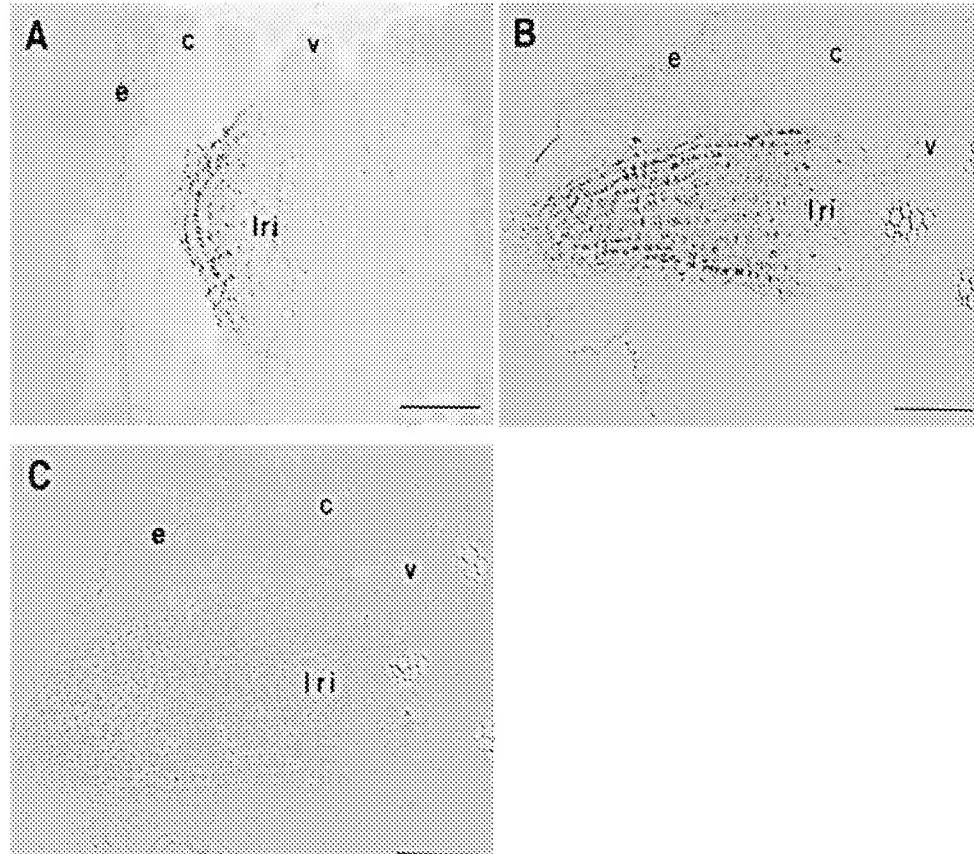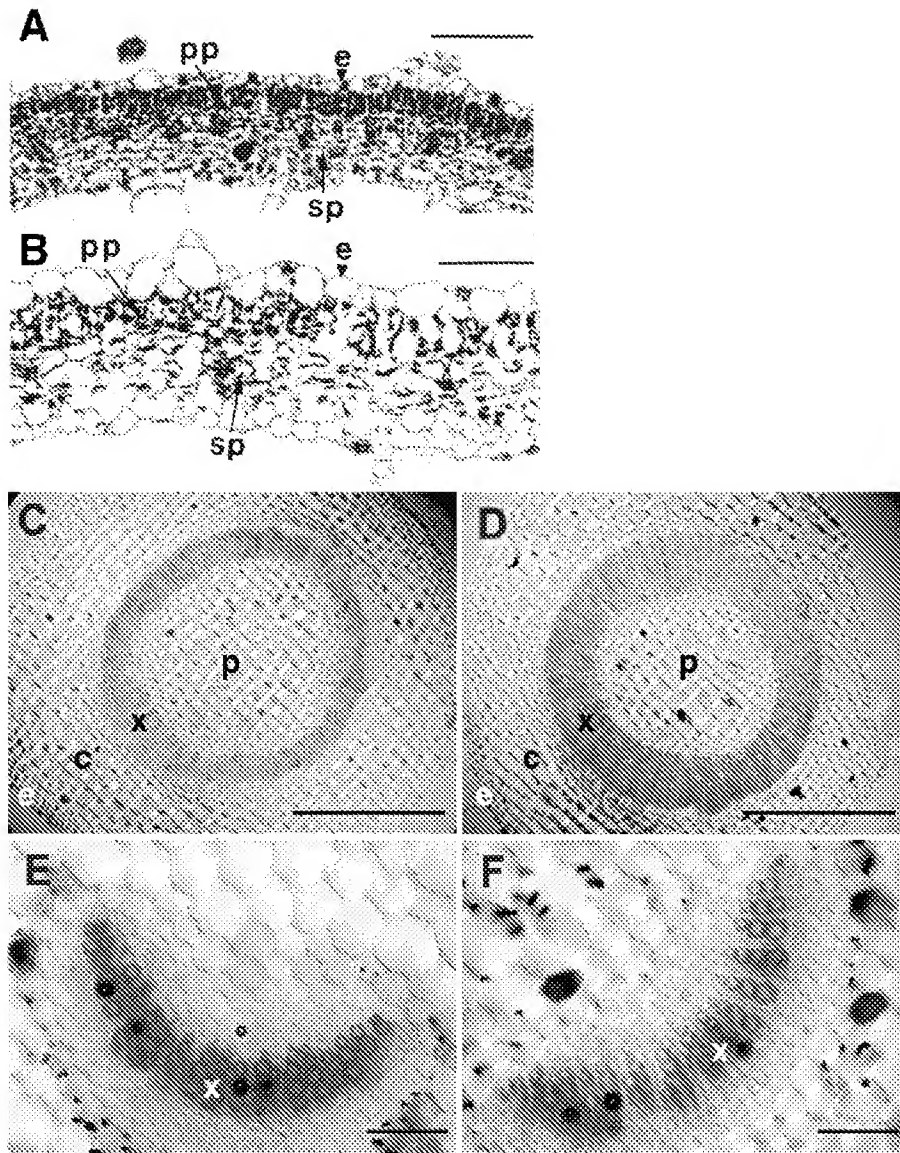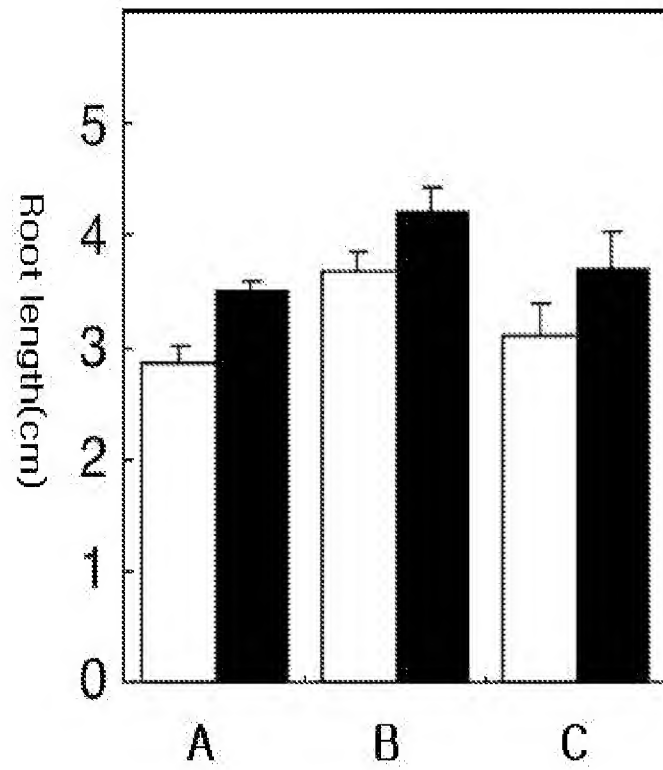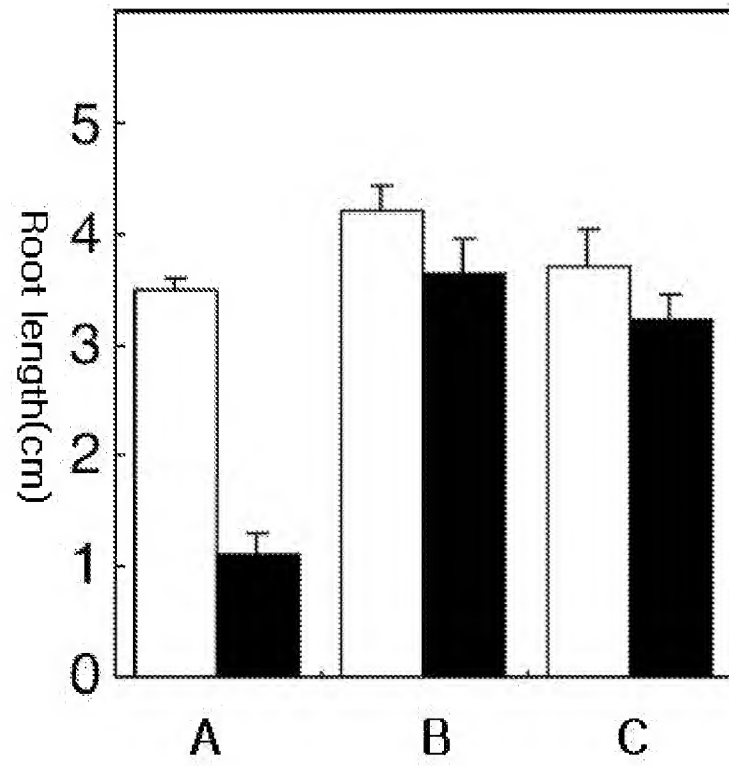gcacgagctt caacctctca tcattaggca ttcagcaagc aagaaaaaaa a   51

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ttc aca atc act acc   tat gcc ttc tca cct tct gga tgg acc   aac gcc   147
Phe Thr Ile Thr Thr Tyr Ala Phe Ser Pro Ser Gly Trp Thr Asn Ala
              20   25   30
cat gcc act ttt tat ggg ggt agt gat gct tca gga act atg ggg gga   195
His Ala Thr Phe Tyr Gly Gly Ser Asp Ala Ser Gly Thr Met Gly Gly
            35   40   45
gct tgt ggg tat ggg aat ctg tat gca act ggg tat gga act aga act   243
Ala Cys Gly Tyr Gly Asn Leu Tyr Ala Thr Gly Tyr Gly Thr Arg Thr
         50   55   60
gca gct tta agc act gcc tta ttt aat gat gga gct tcc tgt ggt cag   291
Ala Ala Leu Ser Thr Ala Leu Phe Asn Asp Gly Ala Ser Cys Gly Gln
65   70   75   80
tgc tac aaa att ata tgt gat tac aaa tca gac tct aga tgg tgc atc   339
Cys Tyr Lys Ile Ile Cys Asp Tyr Lys Ser Asp Ser Arg Trp Cys Ile
                        85   90   95

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Lys Gly Arg Ser Val Thr Val Thr Ala Thr Asn Phe Cys Pro Pro Asn
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Phe Ala Leu Pro Asn Asn Asn Gly Gly Trp Cys Asn Pro Pro Leu Lys
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cat ttt gat atg gcc caa ccc gct tgg gaa aag att ggt att tac aga 483
His Phe Asp Met Ala Gln Pro Ala Trp Glu Lys Ile Gly Ile Tyr Arg
                    130   135   140

gga ggg atc gtc ccc gtg cta ttt caa agg gtt cca tgc aaa aag cat 531
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145   150   155   160

gga ggg gtt agg ttc agt gtg aat ggg agg gac tac ttt gag cta gta 579
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ttg atc agc aat gtg ggg ggt gct gga tcc atc caa tca gtg ttc att 627
Leu Ile Ser Asn Val Gly Gly Ala Gly Ser Ile Gln Ser Val Phe Ile
                      180   185   190

aaa ggc tca aaa act gga tgg atg gca atg tca aga aat tgg ggt tct 675
Lys Gly Ser Lys Thr Gly Trp Met Ala Met Ser Arg Asn Trp Gly Ser
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aat tgg caa tcc aat gcg tat ttg aat ggt caa tct ttg tcc ttc agg 723
Asn Trp Gln Ser Asn Ala Tyr Leu Asn Gly Gln Ser Leu Ser Phe Arg
                    210   215   220

gtc acc acc act gat gga gag acc aga gtt ttc caa gat att gtt cca 771
Val Thr Thr Thr Asp Gly Glu Thr Arg Val Phe Gln Asp Ile Val Pro
225   230   235   240

gta agt tgg aca ttc ggc caa act ttc tct agc cca gtt cag ttc taag 820
Val Ser Trp Thr Phe Gly Gln Thr Phe Ser Ser Pro Val Gln Phe
                      245   250   255

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            35   40   45
Ala Cys Gly Tyr Gly Asn Leu Tyr Ala Thr Gly Tyr Gly Thr Arg Thr
        50   55   60
Ala Ala Leu Ser Thr Ala Leu Phe Asn Asp Gly Ala Ser Cys Gly Gln
        65   70   75   80
Cys Tyr Lys Ile Ile Cys Asp Tyr Lys Ser Asp Ser Arg Trp Cys Ile
                85   90   95
Lys Gly Arg Ser Val Thr Val Thr Ala Thr Asn Phe Cys Pro Pro Asn
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Phe Ala Leu Pro Asn Asn Asn Gly Gly Trp Cys Asn Pro Pro Leu Lys
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His Phe Asp Met Ala Gln Pro Ala Trp Glu Lys Ile Gly Ile Tyr Arg
        130   135   140
Gly Gly Ile Val Pro Val Leu Phe Gln Arg Val Pro Cys Lys Lys His
145   150   155   160
Gly Gly Val Arg Phe Ser Val Asn Gly Arg Asp Tyr Phe Glu Leu Val
                165   170   175
Leu Ile Ser Asn Val Gly Gly Ala Gly Ser Ile Gln Ser Val Phe Ile
            180   185   190
Lys Gly Ser Lys Thr Gly Trp Met Ala Met Ser Arg Asn Trp Gly Ser
        195   200   205
Asn Trp Gln Ser Asn Ala Tyr Leu Asn Gly Gln Ser Leu Ser Phe Arg
        210   215   220
Val Thr Thr Thr Asp Gly Glu Thr Arg Val Phe Gln Asp Ile Val Pro
225   230   235   240
Val Ser Trp Thr Phe Gly Gln Thr Phe Ser Ser Pro Val Gln Phe
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4

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cucuggucuc uccaucagug guggugaccc ugaaggacaa agauugacca uucaaauacg 180
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auucggccaa acuuucucua gcccaguuca guucuaagcu gauuacagau aaaccaacca 240
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